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Supplemental Information

A Conditional Dependency on MELK for the Proliferation of Triple-Negative Breast Cancer Cells

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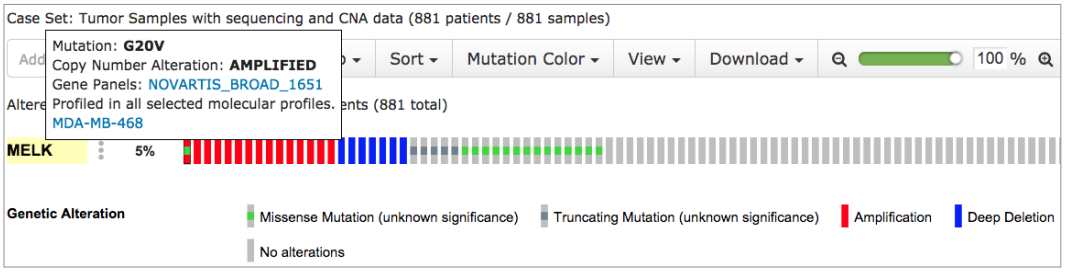
A



B

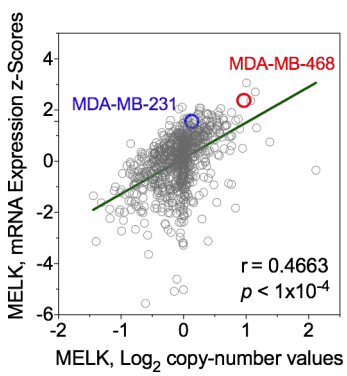


Figure S1. Genetic Alteration of MELK Gene in Human Cancer Cell Lines, related to Figure 3 and 4.

(A) Status of MELK genetic alteration in human cancer cell lines. The amplification, deletion, and/or mutation occur in 5% human cell lines (total 881 lines tested; Barretina et al., 2012). The oncoprint visualization was derived from the inquiry of MELK gene in the database of Cancer Cell Line Encyclopedia (Barretina et al., 2012), performed at cBioPortal for Cancer Genomics (www.cbioportal.org; Cerami et al., 2012; Gao et al., 2013). Note that MDA-MB-468, one of TNBC lines we previously used for tet-on inducible MELK knockdown (Wang et al., 2014) harbors a splice site mutation (G20V) and gene amplification of MELK. Given that multiple cutting derived from CRISPR guides targeting amplified loci causes DNA damage response and consequently gene-independent inhibition of cancer cell growth (Aguirre et al., 2016; Munoz et al., 2016), MDA-MB-468 is considered less suitable for assessing MELK function using CRISPR/Cas9-mediated gene editing.

(B) Expression of MELK (assayed by microarray) versus its copy number (log_2) in cancer cell lines. The data were generated by Cancer Cell Line Encyclopedia (CCLE) (Barretina et al., 2012) and downloaded from cBioPortal for Cancer Genomics. Note that MDA-MB-231 cell lines does not have MELK gene amplification, and have a relatively high level of MELK expression.

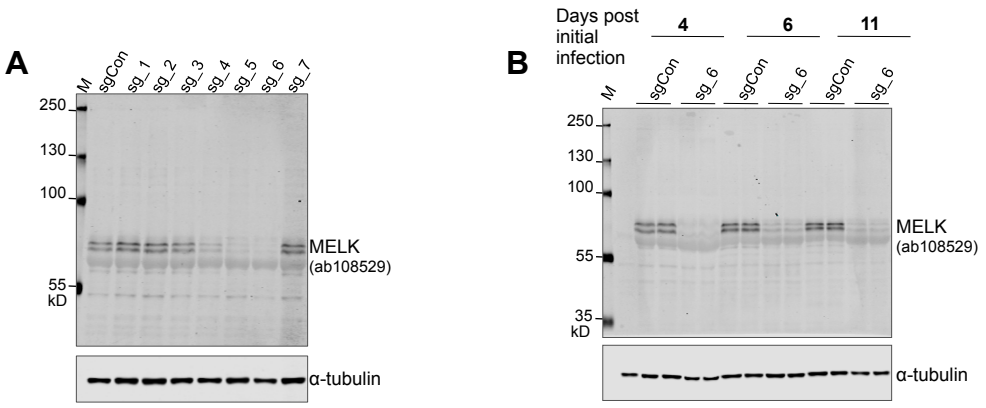


Figure S2. Identifying Guide Sequences Targeting MELK, related to Figure 2.

(A) Fluorescent western blotting analysis of MELK in MDA-MB-468 cells infected with control or MELK-targeting lentiCRISPR. Cells were harvested seven days post infection. Total cell lysates were resolved on 8% SDS-PAGE, transferred onto nitrocellulose membranes. Membranes were incubated with anti-MELK (ab108529, Abcam). The images were acquired via Odyssey CLxinfrared imaging system (LI-COR Biosciences). Note that in this cell line, sg_6 is the most effective guide sequence in reducing MELK protein abundance.

(B) Examining the efficiency of gene editing in cells harvested at different time points after lentiCRISPR infection.

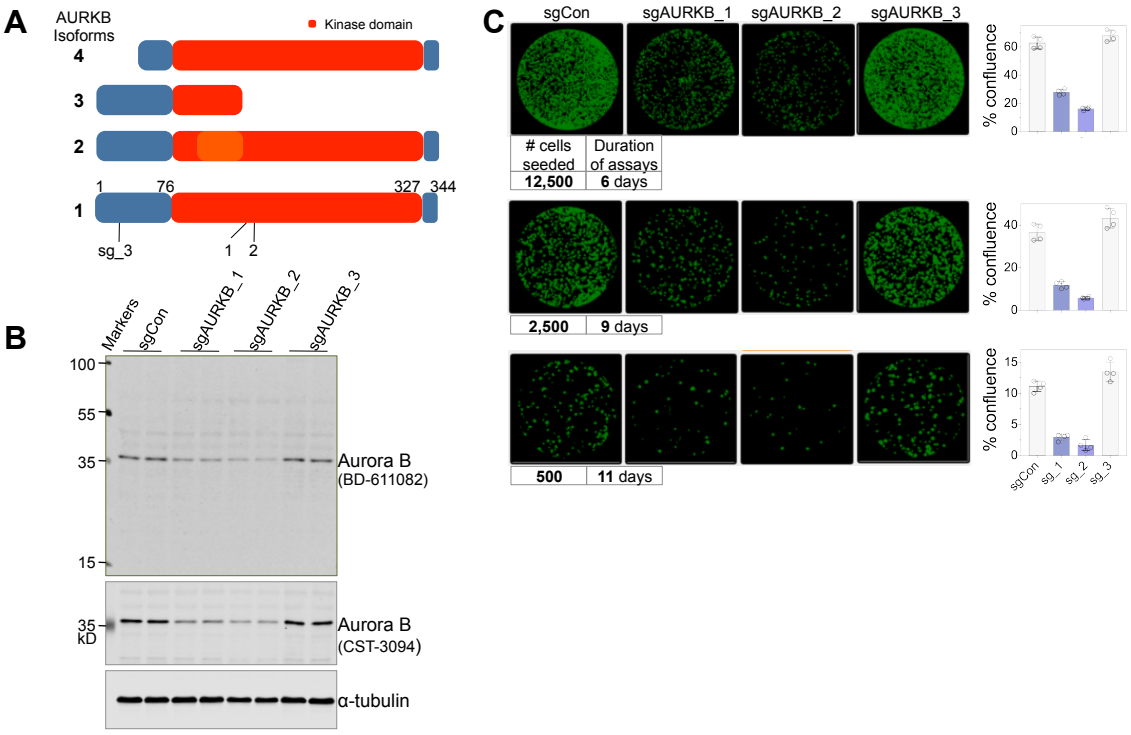


Figure S3. LentiCRISPR-mediated Editing of AURKB in TNBC Cells, related to Figure 5.

(A) Schematic diagram of human AURKB transcripts. The longest full-length one is isoform 1, shown at the bottom. The target locations of three guide sequences are indicated.

(B) Fluorescent western blotting analysis of AURKB in MDA-MB-468 cells infected with control or lentiCRISPR targeting AURKB (harvested five days after initial infection). Information of AURKB antibodies is indicated

(C) LentiCRISPR-mediating gene editing of AURKB suppresses the growth of cancer cells in a manner that is largely independent of the duration of cell growth assays. Four days after the initial virus infection, cells were harvested and seeded in 24-well plates. Cell proliferation was measured by Celigo Image Cytometry (Nexcelom Bioscience) on indicated days post plating (mean ± SD; n=4). The whole-well images filled in with a green color indicate cell confluence.

TRANSPARENT METHODS

List of antibodies and reagents for immunoblotting

	SOURCE	IDENTIFIER	DESCRIPTION
PageRule Plus Prestained Protein Ladder	ThermoFisher Scientific	26619	Generating near-infrared signal in fluorescence immunoblotting.
Anti-MELK	Abcam	ab108529	Rabbit monoclonal (EPR3981)
	Cell Signaling	2274	Rabbit polyclonal
	Bethyl	A303-136A	Rabbit polyclonal
	R&D	AF4820	Sheep polyclonal
Anti-AURKB	Cell Signaling	3094	Rabbit polyclonal
	BD Biosciences	611082	Mouse monoclonal (6)
Anti-PLK1	Cell Signaling	4513	Rabbit monoclonal (208G4)
Anti-RAS	Cell Signaling	8955	Rabbit monoclonal (D2C1)
Anti-MYC	Abcam	ab32072	Rabbit monoclonal (Y69)
Anti-α-tubulin	Cell Signaling	3873	Mouse monoclonal (DM1A)
Anti-rabbit IgG	Life Technologies	A21109	Secondary antibody; conjugated with Alexa Fluor 680
Anti-mouse IgG	Rockland	610-145-121	Secondary antibody; conjugated with DyLight 800
Anti-Sheep IgG	Jackson ImmunoResearch	713-655-147	Secondary antibody; conjugated with Alexa Fluor 790

Cell Culture

Human breast cancer cell lines (BT549, MDA-MB-231, MDA-MB-468, and MCF7) were cultured in RPMI 1640 medium (Invitrogen) supplemented with 10% fetal bovine serum (FBS, Gemini Bioproducts) and 1% penicillin/streptomycin (Invitrogen). For cells stably introduced with tetracyclin-inducible shRNAs, Tet System Approved FBS (Clontech, cat# 631106) was used.

HEK293T cells were cultured in DMEM (Invitrogen) with 10% FBS and 1% penicillin/ streptomycin.

Construction of all-in-one lentiCRISPR vectors

Cloning of lentiCRISPR targeting MELK, PLK1, AURKB, KRAS, or MYC was performed in the backbone of all-in-one lentiCRISPR v2 vector (a gift from Feng Zhang, Addgene plasmid # 52961; Sanjana et al., 2014). Briefly, forward and reverse oligos (synthesized at Eton Bioscience) were mixed and annealed, ligated with BsmBI-digested lentiCRISPR backbone, and then transformed into competent E.coli (Stbl3). Following overnight incubation in warm room, single bacterial colonies were transfered into 50 µl sterile water, 2 ul of the suspension was then used as template for PCR using U6 primer and individual reverse oligo as primers. Positive colonies were subject to culture for midiprep of plasmids (Qiagen). Plasmids were verified by sequencing using U6 primer (Eton Bioscience). The sequence of oligos is listed in the following table.

Guide RNA sequences	
sgCon	GAGCTGGACGGCGACGTAAA
sgMELK_1	ATGAATTACATGAAACTATT
sgMELK_2	AACCCGATGTGGTGGGTATC
sgMELK_3	TATGAATTACATGAAACTAT
sgMELK_4	TCAATCTCCGTTTTGATCCG
sgMELK_5	CCGGATCAAAACGGAGATTG
sgMELK_6	CTATCTGACGGAAGACAACC
sgMELK_7	AGCGGCTTAAGGGCGATGCC
sgAURKB_1	ATTCTAGAGTATGCCCCCCG
sgAURKB_2	GCTCCTTGTAGAGCTCCCCG
sgAURKB_3	GCTCTTTCCGGAGGACTCGC
sgPLK1_1	AGCCAAGCACAATTTGCCGT
sgPLK1_2	TACCTACGGCAAATTGTGCT
sgKRAS	AACATCAGCAAAGACAAGAC
sgMYC	GCCGTATTTCTACTGCGACG

Lentivirus Packaging and Infection

One day prior to transfection (day 0), HEK293T cells were seeded in T-25 tissue culture flasks (2.5 - 3 million cells seeded). On day 1, 4 µg DNA (2 µg vector DNA, 1.5 µg pCMVdR8.91, and 0.5 µg pMD2-VSVG) and 12 µl polyethylenimine (PEI; homemade from powder purchased from Polysciences, cat # 23966-2) were each diluted in PBS, mixed, and added to cells following 15 min incubation at room temperature. The medium was refreshed on day 2. On day 3 and 4, viral supernatant was collected and filtered through 0.45-µm membranes, supplemented with polybrene at the final concentration of 8 µg/ml (Millipore, cat# TR-1003-G), and then freshly added to target cells.

Target cells were seeded in 6-well plates one day after HEK293T transfection. On the next day, after removal of old medium, 1.5 - 2 ml fresh viral supernatant collected was added to the cells. The infection was repeated on the following day. Two days after the initial infection, cells were refreshed with medium containing puromycin (1.5 μg/ml). After two days of puromycin selection, all uninfected cells (set as a control) are expected to die while infected cells appear as healthy as normally cultured cells.

PCR and sequencing

Genomic DNA was extracted using a PureLink Genomic DNA Mini Kit (Invitrogen, #K1820-01), and used as template for PCR. NovaTaq™ Hot Start DNA Polymerase (EMD Millipore, #71091) was used to amplify exon 5 of human MELK, with the following primers: Forward, 5'-CCTTACTCGGTTCCATTCCCT-3'; reverse, 5'-AGGTATGACTGGAGCAACAACA-3'. The forward primer was also used for Sanger sequencing (Eton Bioscience).

Immunoblotting

Cells were rinsed with PBS and then lysed in 1x SDS-PAGE sample buffer (typically 200-400 μl used for one well of cells that were seeded one day earlier at the density of 4×10^5 per well of a 6-well plate). Lysates were boiled for 5 min before loading on 8% for detection of MELK, MYC, PLK1, or α-tubulin, or 12% SDS-PAGE for detection of AURKB or KRAS. PageRuler™ Plus Prestained Protein Ladder (ThermoFisher Scientific, cat# 26619) was loaded (1 μl in the total volume of 20 μl SDS-PAGE sample buffer) (Note that the total six blue-prestained recombinant proteins fluoresce in the 700 nM channel of Odyssey Imaging System). Nitrocellulose membrane with protein transferred was blocked with 5% non-fat milk and was then incubated with primary antibodies overnight at 4°C. After washing, the membrane was incubated with fluorophore-conjugated secondary antibodies for one hour at room temperature. The membrane was then washed and scanned with an Odyssey CLxinfrared imaging system (LI-COR Biosciences). Antibodies are listed in a separate table.

Cell growth assays

After cells were harvested and re-suspended in medium, cell counting was performed with the Countess Automated Cell Counter (Life Technologies). Cell viability, determined via trypan blue dye exclusion, is expected to be higher than 90% in all assays. A high concentration of cell suspension was first made (e.g. 5×10^4 cell per ml), and then diluted serially (e.g. 5-fold

dilutions involve mix 1 ml concentrated cells with 4 ml medium). Cells were subsequently seeded in multi-well plates (typically, 1 and 0.5 ml per well for 12- and 24-well plates respectively).

For colongenic assays, each well was replenished with medium five days after seeding. Cell proliferation was measured by calculating cell confluence, which was performed via scanning whole wells with Celigo Image Cytometry (Nexcelom Bioscience). Alternatively, at the endpoints of assays, cells were fixed with formalin and subsequently stained with crystal violet. The stained plates were scanned before the staining was extracted by 10% acetic acid with absorbance measured.

SUPPLEMENTAL REFERENCES

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